

1   **TITLE**

2

3   Use of the disorazoles and their derivatives for the treatment of benign and malignant  
4   oncoses

5

6   **BACKGROUND**

7

8   For the next few years, a dramatic increase in oncoses and tumor-related  
9   cases of death is expected worldwide. In 2001, worldwide approximately 10  
10   million people were suffering from cancer and over 6 million people died from  
11   this disease. The development of tumors is a fundamental disease of higher  
12   organisms in the plant kingdom, in the animal kingdom and in humans. The  
13   generally recognized multistep model of carcinogenesis assumes that as a  
14   result of accumulation of a number of mutations in an individual cell this is so  
15   modified in its proliferation and differentiation behavior that finally, via benign  
16   intermediate stages, a malignant state with metastasis is reached. The term  
17   cancer or tumor conceals a clinical picture with more than 200 various  
18   individual diseases. Oncoses can proceed in a benign or malignant manner.  
19   The most important tumors are those of the lung, the breast, the stomach, the  
20   neck of the uterus, the prostate, the head and neck, the large and small  
21   intestine, the liver and the blood system. There are great differences with  
22   respect to course, prognosis and therapy behavior. More than the 90% of the  
23   cases recognized relate to solid tumors, which in particular in the advanced

1 stage or on metastasis are treatable with difficulty or are untreatable. The three  
2 pillars of cancer control are still surgical removal, irradiation and chemotherapy.  
3 In spite of great advances it has not yet been possible to develop medicaments  
4 which bring about a marked prolongation of the survival time or even a  
5 complete cure in the widespread solid tumors. It is therefore meaningful to  
6 invent novel medicaments for the control of cancer.

7

## 8 **DESCRIPTION OF THE INVENTION**

9

10 The present invention relates to disorazole – with the exception of disorazole  
11 A1 - and derivatives of the disorazoles, and to their use as medicaments, in  
12 particular for the treatment of benign and malignant tumors in humans and  
13 mammals.

14

15 It has now surprisingly been found that the disorazoles E1 and D1 in particular  
16 possess an outstanding cytotoxic action on various human tumor cell lines. In  
17 nano- and picomolar concentrations, the division, inter alia, of ovarian  
18 carcinoma, prostate carcinoma, glioblastoma, lung carcinoma and breast  
19 cancer cells is inhibited. The action of the disorazoles E1 and D1 is in this case  
20 cell cycle-dependent, even in nanomolar concentrations the cell cycle is held in  
21 the G2/M phase and the cancer cells are forced

22

1 into apoptosis. It has further been possible to show that the antiproliferative  
2 action of the disorazoles claimed is based, inter alia, on an effective inhibition  
3 of tubulin polymerization. Disorazole E1 is in particular also highly active  
4 against paclitaxel- and vindesine-resistant cell lines. It was inventively possible  
5 to show that disorazole E1 is highly potent with respect to biological action and  
6 thus use as an active compound in a medicament for the control of cancers is  
7 possible.

8  
9 This matters in particular, since disorazole A1 is unsuitable for use as a  
10 cytostatic (G. Hoefle, annual report 1999/2000 of the Gesellschaft für  
11 Biotechnologische Forschung [Association for Biotechnological Research]  
12 GBF, p. 103).

13  
14 In a therapeutic experiment, using, for example, NCI-H460 tumor xenograft-  
15 bearing nude mice – but not restricted thereto - it was possible to observe,  
16 however, for disorazole E1 administered i.v, a significant reduction in tumor  
17 growth even at doses which produced no weight decrease or perhaps even  
18 mortality.

19  
20 Natural substances are an important source for novel lead structures in  
21 pharmaceutical research and are in some cases also directly suitable for the  
22 development of a novel medicament (Y.-Z. Shu, J. Nat. Prod., 1998, 61, 1053-

1 1071). It is known that many natural substances possess strongly cytotoxic  
2 action (V. J. Ram, S. Kumari, DNP, 2001, 14(8), 465-482).

3 It is known that natural substances of the group consisting of the disorazoles  
4 are isolated from the bacterium of the strain Sorangium cellulosum So ce12 (R.  
5 Jansen, H. Irschik, H. Reichenbach, V. Wray, G. Höfle, Liebigs Ann. Chem.,  
6 1994, (8), 759-773). In total, 29 disorazoles have been isolated and  
7 characterized physicochemically. For the disorazole A1, it was reported that it  
8 possesses an antiproliferative action in cell models (H. Irschik, R. Jansen, K.  
9 Gerth, G. Höfle, H. Reichenbach, J. Antibiot. 1995, 48(1), 31-35; Y. A.  
10 Elnakady, Dissertation, T.U. Braunschweig, 2001). Use for the treatment of  
11 oncoses was, however, neither disclosed nor suggested. A biological  
12 investigation of the other disorazoles was not carried out.

13

14 The compounds according to the invention are suitable, without being restricted  
15 thereto, for employment as medicaments for the treatment of benign and  
16 malignant oncoses or

17 other antiproliferative disorders in humans and animals. In principle, the  
18 compounds according to the invention are suitable for the control of all  
19 disorders which are based on the uncontrolled and rapid division of cells and  
20 thereby cause pathological conditions. The compounds according to the  
21 invention can be employed as an individual substance or in combination with  
22 further cytotoxic substances, e.g. cisplatin, carboplatin, doxorubicin, ifosfamide,  
23 cyclophosphamide, 5-FU, methotrexate and in particular in combination with

1 inhibitors of signal transduction, such as, for example, Herceptin, Glivec or  
2 Iressa, but not restricted thereto.

3

4 Synthetic and semisynthetic analogs of the disorazoles also possess  
5 antiproliferative action. By means of specific modification of the molecular  
6 shape, important properties such as biological inhibitory action, stability and  
7 biophysical properties can be modulated. In this manner, therapeutically  
8 valuable derivatives of the starting compounds are obtainable. A further aim of  
9 the derivatization consists in moderating possible toxic side effects.

10

11 The compounds according to the invention can be administered as liquid  
12 pharmaceutical forms. This is carried out in the manner suitable in each case in  
13 the form of solutions or suspensions.

14

15 The compounds according to the invention can be administered in a suitable  
16 administration form, preferably into an artery, intraarterially as an injection; into  
17 a vein, intravenously as an injection or infusion; into the skin, intracutaneously  
18 as an injection; under the skin, subcutaneously as an injection; into the muscle,  
19 intramuscularly as an injection; into the abdominal cavity, intraperitoneally as  
20 an injection or infusion.

21

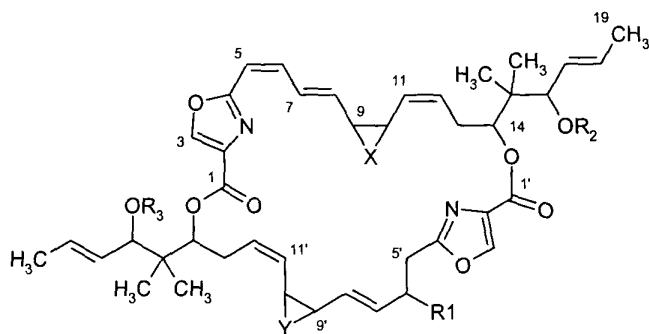
22 If the compounds of the general formula I according to the invention have at  
23 least one asymmetric center, they can be present in the form of their

racemates, in the form of the pure enantiomers and/or diastereomers or in the form of mixtures of these enantiomers and/or diastereomers, namely both in substance and as pharmaceutically acceptable salts of these compounds. The mixtures can be present in any desired mixing ratio of the stereoisomers.

If possible, the configurations of each of the double bonds in the compounds according to the invention can independently of one another in each case be E or Z.

If possible, the compounds according to the invention can be present in the form of the tautomers.

According to one embodiment, the invention relates to compounds of the general formula I:



Formula I

in which independently of one another

1 R1 is:

- 2 (i) hydrogen
- 3 (ii) OR4
- 4 (iii) part of a double bond to C5'

5

6 R2, R3 and R4 are:

7

- 8 (i) hydrogen
- 9 (ii) unsubstituted or substituted (C<sub>1</sub>-C<sub>6</sub>)-alkyl,
- 10 (iii) (C<sub>1</sub>-C<sub>4</sub>)-alkyl substituted by one or more fluorine atoms,  
11 preferably a trifluoromethyl group,
- 12 (iv) unsubstituted or substituted (C<sub>1</sub>-C<sub>4</sub>)-alkyl-(C<sub>6</sub>-C<sub>14</sub>)-aryl,  
13 unsubstituted or substituted (C<sub>1</sub>-C<sub>4</sub>)-alkyl-heteroaryl,
- 14 (v) (C<sub>1</sub>-C<sub>4</sub>)-alkoxycarbonyl, (C<sub>1</sub>-C<sub>4</sub>)-alkylaminocarbonyl (C<sub>1</sub>-C<sub>4</sub>)-  
15 alkylaminothiocarbonyl, (C<sub>1</sub>-C<sub>6</sub>)-alkyl-carbonyl or (C<sub>1</sub>-C<sub>6</sub>)-  
16 alkoxycarbonyl-(C<sub>1</sub>-C<sub>6</sub>)-alkyl,

17

18 it being possible for the substitution of the alkyl radical by F, Cl,  
19 Br, I, CN, NH<sub>2</sub>, NH-(C<sub>1</sub>-C<sub>20</sub>)-alkyl, NH-(C<sub>3</sub>-C<sub>12</sub>)-cycloalkyl, OH, O-  
20 (C<sub>1</sub>-C<sub>20</sub>)-alkyl to take place singly or,

21

22 on identical or different atoms, multiply by identical or different  
23 substituents, and it being possible for the substitution of an aryl

1 radical by F, Cl, Br, I, CN, NH<sub>2</sub>, NH-(C<sub>1</sub>-C<sub>20</sub>)-alkyl, OH, O-(C<sub>1</sub>-C<sub>20</sub>)-  
2 alkyl and/or (C<sub>3</sub>-C<sub>8</sub>)-heterocyclyl having 1 to 5 heteroatoms,  
3 preferably nitrogen, oxygen, sulfur to take place singly or, on  
4 identical or different atoms, multiply by identical or different  
5 substituents,

6

7 and

8

9 X, Y are: in each case individually independently of one another or  
10 together oxygen, sulfur, two vicinal hydroxyl groups, two vicinal  
11 methoxy groups, part of a double bond,

12

13 a compound being excluded in which R<sub>1</sub> is methoxy, R<sub>2</sub>, R<sub>3</sub> are hydrogen, X is  
14 oxygen and Y is the part of a double bond.

15

16 The term „aryl“ means for the purpose of this invention aromatic hydrocarbons,  
17 inter alia phenyls, naphthyls and anthracenyls. The radicals may also be fused  
18 to other saturated, (partially) unsaturated or aromatic ring systems.

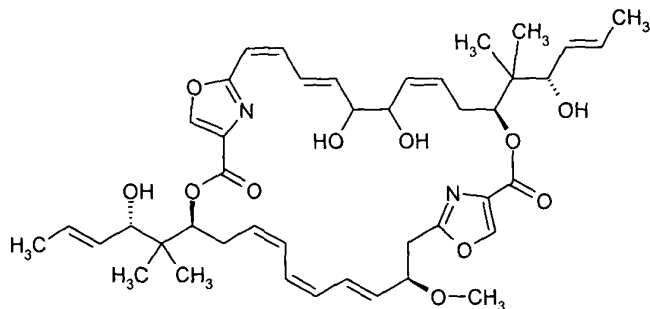
19

20 The term „heteroaryl“ stands for a 5-, 6- or 7-membered cyclic aromatic radical  
21 which comprises at least 1, where appropriate also 2, 3, 4 or 5, heteroatoms,  
22 the heteroatoms being identical or different.

23







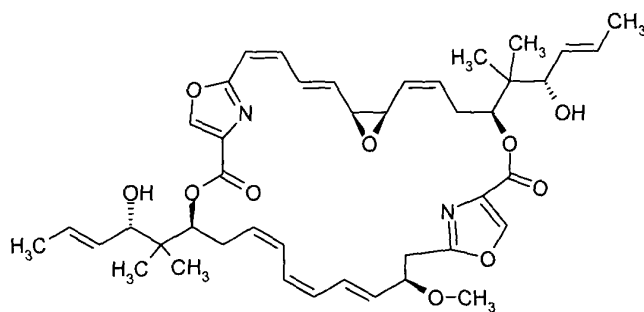
Formula III: disorazole D1

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4 (3) Disorazole A1 is expressly not a subject of this invention.



Formula IV: disorazole A1

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8 The invention will be illustrated in greater detail with the aid of the following  
9 examples, without being restricted thereto.

10

11

### Examples

12

#### Use possibilities

13

1    Example 1

2    Disorazoles such as, for example, disorazole E1 are preferred as an active  
3    compound in a ready-to-use medicament for the treatment of malignant  
4    oncoses such as breast cancer, lung cancer, ovarian cancer, skin cancer,  
5    prostate cancer, colonic cancer, renal cell cancer, hepatic cancer, pancreatic  
6    cancer and cancers of the brain.

7    In a preferred administration form, the active compound is present as a  
8    lyophilizate together with the excipients known to the person skilled in the art in  
9    an injection bottle and is dissolved using physiological saline solution before  
10   use, then diluted in an injection bag and administered to the patient with the aid  
11   of a cannula into the vein. The dose, depending on the stage of the oncosis  
12   and the state of health of the patient, is between 0.1 mg and 100 mg of active  
13   compound per m<sup>2</sup>. The infusion period depends on the objective criteria of the  
14   disease.

15

16   Example 2

17   Use of disorazoles such as, for example, disorazole E1 as an active compound  
18   in a ready-to-use medicament for the treatment of inflammatory diseases.  
19   These include, for example, inflammatory airway diseases such as bronchial  
20   asthma, allergic rhinitis, allergic conjunctivitis, atopic dermatitis, eczema,  
21   allergic angitis, inflammations mediated by eosinophils such as eosinophilic  
22   pneumonia and PIE syndrome (pulmonary infiltration with eosinophilia),

1 urticaria, ulcerative colitis, Crohn's disease and proliferative skin diseases such  
2 as psoriasis and keratosis.

3

#### 4 Example 3

5 Use of disorazoles such as, for example, disorazole E1 as an active compound  
6 in a ready-to-use medicament having immunomodulatory action for the  
7 treatment of immune and autoimmune diseases. Such diseases can include,  
8 for example, joint inflammations such as arthritis and rheumatoid arthritis and  
9 other arthritic diseases such as rheumatoid spondylitis and osteoarthritis.  
10 Further possibilities of use are the treatment of patients who are suffering from  
11 sepsis, septic shock, Gram-negative sepsis, toxic shock syndrome, respiratory  
12 distress syndrome, asthma and other chronic pulmonary diseases, bone  
13 resorption diseases or transplant rejection reactions or other autoimmune  
14 diseases, such as lupus erythematosus, multiple sclerosis, glomerulonephritis  
15 and uveitis, insulin-dependent diabetes mellitus and chronic demyelination.

16

#### 17 Example 4

18 Use of disorazoles such as, example disorazole E1 as an active compound in a  
19 ready-to-use medicament which can be employed for the therapy of infections  
20 such as virus infections and parasite infections, for example for the therapy of  
21 malaria, infection-related fever, infection-related muscle pain, HIV infections  
22 (AIDS) and cachexias.

23

1    **Production**

2

3    For the administration of the compounds according to the invention, parenteral,  
4    transdermal, topical, inhalative and intranasal preparations are preferably  
5    suitable.

6

7    The production, filling and sealing of the preparations is carried out under the  
8    customary antimicrobial and aseptic conditions.

9

10   In addition to at least one constituent according to the invention, the  
11   pharmaceutical forms, depending on the pharmaceutical form employed,  
12   optionally contain excipients, such as, inter alia, solvents, solution accelerators,  
13   solubilizers, emulsifiers, wetting agents, antifoams, gel-forming agents,  
14   thickeners, buffers, salt-forming agents, preservatives, antioxidants, colorants,  
15   taste and odor corrigents. The choice of the excipients and the amounts thereof  
16   to be employed depends on the pharmaceutical form chosen and is adapted to  
17   the formulations known to the person skilled in the art.

18

19   The medicaments according to the invention can be administered in a suitable  
20   administration form to the skin, epicutaneously as a solution, suspension,  
21   emulsion, foam, ointment, paste or patch; via the nasal mucosa, nasally as  
22   drops, ointment, or spray; via the bronchial and alveolar epithelium, pulmonarily  
23   or by inhalation as an aerosol or inhalant; via the conjunctiva, conjunctivally as

1 eye drops, eye ointment, eye tablets, lamellae or eye lotion; into an artery,  
2 intraarterially as an injection; into a vein, intravenously as an injection or  
3 infusion, paravenously as an injection or infusion; into the skin,  
4 intracutaneously as an injection or implant; under the skin, subcutaneously as  
5 an injection or implant; into the muscle, intramuscularly as an injection or  
6 implant; into the abdominal cavity, intraperitoneally as an injection or infusion.

7

8 In tumor therapy, the compounds of the general formula I according to the  
9 invention can be employed as an individual substance or in combination with  
10 further cytotoxic substances, such as, for example, paclitaxel, docetaxel,  
11 vincristine, vindesine, cisplatin, carboplatin, doxorubicin, ifosfamide,  
12 cyclophosphamide, 5-FU, methotrexate or in combination with  
13 immunomodulators or antibodies and in particular in combination with inhibitors  
14 of signal transduction, such as, for example, Herceptin, Glivec or Iressa.

15

#### 16 Example 5

17 Preparations for the parenteral administration of disorazoles such as, for  
18 example, disorazole E1, can be present in separate dose unit forms such as,  
19 for example, ampoules or vials. Preferably, solutions of the active compound  
20 are used, preferably aqueous solutions and especially isotonic solutions or  
21 alternatively suspensions. These injection forms can be made available as a  
22 ready-to-use preparation or are prepared only directly before use by mixing the

1 active compound, for example the lyophilizate, if appropriate with further solid  
2 carriers, with the desired solvent or suspending agent.

#### 3 4 Example 6

5 Preparations for the intranasal administration of disorazoles such as, for  
6 example, disorazole E1, can be present as aqueous or oily solutions or as  
7 aqueous or oily suspensions. They can also be present as lyophilizates, which  
8 are prepared before use using the suitable solvent or suspending agent.

#### 9 10 Biological actions of the compounds according to the invention

#### 11 12 Example 7 Antiproliferative action on various tumor cell lines

13  
14 The compounds according to the invention were investigated for their  
15 antiproliferative activity in a proliferation test on established tumor cell lines  
16 (D.A. Scuderio et al. Cancer Res. 1988, 48, 4827-4833). The test used  
17 determines the cellular dehydrogenase activity and makes possible a  
18 determination of the cell vitality and indirectly of the cell count. The cell lines  
19 used are the human cervical carcinoma cell line KB/HeLa (ATCC CCL17), the  
20 ovarian adenocarcinoma cell line SKOV-3 (ATCC HTB77), the human  
21 glioblastoma cell line SF-268 (NCI 503138), the lung carcinoma cell line NCI-  
22 H460 (NCI 503473) and the human colon adenocarcinoma cell line RKOP 27.

1 The cytotoxic or growth-inhibiting activity of the compounds described is shown  
 2 in table 1. The results show a very potent inhibition of the proliferation of  
 3 selected tumor cell lines by the substances mentioned.

4  
 5

Example	XTT proliferation assay, EC50 in [ $\mu$ g/ml]				
	KB/Hela	SKOV3	SF-268	NCI-H460	RKOP 27
Disorazole E1	0.00007	0.00002	0.00017	0.00004	0.00006
Disorazole D1	<0.0001	<0.0001	0.00035	<0.0001	0.0003
Disorazole A1	0.00015	0.0002	0.00027	0.00015	0.00025
Paclitaxel	0.01	0.01	0.01	0.01	
Vindesine	0.002	0.002	0.005	0.006	

6

7 Table 1: Inhibition of proliferation by substances according to the  
 8 invention in the XTT cytotoxicity test on human tumor cell lines

9

# 10 **Example 8 Antiproliferative action on MDR tumor cell lines**

11

12 For further characterization, the substances according to the invention were  
 13 investigated against multi-drug-resistant cell lines (MDR) in comparison to the  
 14 nonresistant wild-type cell lines. The cell lines investigated are the acute  
 15 myeloid leukemia cell line LT1 and the resistant line LT12/mdr. Moreover, the



1 murine P388 cell line (methylcholanthrene-induced lymphoid neoplasm) and  
2 the doxorubicin-resistant P388 were used as test systems.

3

4 The results are shown in summarized form in table 2 below:

5

Substance	XTT proliferation assay, EC <sub>50</sub> in [µg/ml]			
	LT12	LT12MDR	P388	P388ADR
Disorazole E1	0.0001	0.004	0.0004	0.001
Paclitaxel	0.005	0.340	0.035	>3.16
Vindesine	0.0009	0.222	0.009	0.94

6

7 Table 2: Inhibitory action of disorazole E1 and reference substances in the  
8 XTT proliferation test on nonresistant and resistant tumor cell  
9 lines.

10

11 Disorazole E1 shows a very potent inhibitory action on all cell lines tested,  
12 while in the case of the classical tubulin inhibitors such as paclitaxel or  
13 vincristine a greatly decreased action and cross resistances to the MDR1 cell  
14 lines can be detected.

15

16 **Example 9** Inhibition of the polymerization of tubulin

17

1 The substances were tested in an in-vitro test for inhibition of the  
2 polymerization of bovine  $\beta$ -tubulin (D.M. Bollag et al. Cancer Res. 1995, 55,  
3 2325-2333). In this test, tubulin purified by cycles of polymerization and  
4 depolymerization is employed, and is polymerized by addition of GTP and  
5 warming. The EC<sub>50</sub> values of the inhibition of polymerization of  $\beta$ -tubulin with  
6 and without 30% associated proteins (MAPs) are indicated in table 3.

7

Substances	EC <sub>50</sub> in [ $\mu$ g/ml]
	with 30% MAPs
Disorazole E1	1.50
Disorazole D1	2.50
Disorazole A1	4.80
Vindesine	0.40

8 experiments: n=2

9

10 Table 3: Inhibition of the polymerization of  $\beta$ -tubulin with 30% MAPs.

11

12 The results show that the disorazoles E1 and D1 inhibit tubulin polymerization  
13 at low concentrations.

14

15

16

17

1    **Example 10 Cell cycle analysis**

2

3    The cell cycle comprises the development of the cell from one cell generation  
4    to the next. During the resting phase (G0) and presynthetic phase (G1), the  
5    cell has a diploid chromosome set (2c). In the synthesis phase (S), the amount  
6    of DNA is increased by replication. The S phase ends by reaching the  
7    premitotic phase (G2M), in which the cell has a reduplicated chromosome  
8    complement (4c) and doubled DNA content. In the subsequent, transient  
9    mitosis phase (M) the uniform division of the reduplicated chromosomes to two  
10    daughter cells occurs, which then in each case again show a diploid DNA  
11    content and are in the G01 phase, so that the cell cycle can begin anew.

12

13    For the cell cycle analysis, KB/HeLa cells were treated with the test substances  
14    in different concentrations (0.1-1000 nM) for 24 hours at 37°C.

15

16    The percentage proportion of the cells arrested in the G2/M phase of the cell  
17    cycle after treatment with reference substances or selected test substances is  
18    shown in table 4 below. The results were evaluated using special analysis  
19    software (ModFit™).

20

1

Example	EC <sub>50</sub> in [nM] (50% cells in G2/M)
Disorazole E1	1.6
Paclitaxel	46
Vindesine	3.0

2

3 Table 4: concentration at which 50%  
4 of the cells are arrested in the G2/M phase.

5

6

7 The compounds according to the invention have the highest activities in  
8 comparison with the reference compounds. In particular, disorazole E1 inhibits  
9 the cell cycle in the G2/M phase in extremely low concentrations.

10

11

### 12 **Example 11 *In vivo* results**

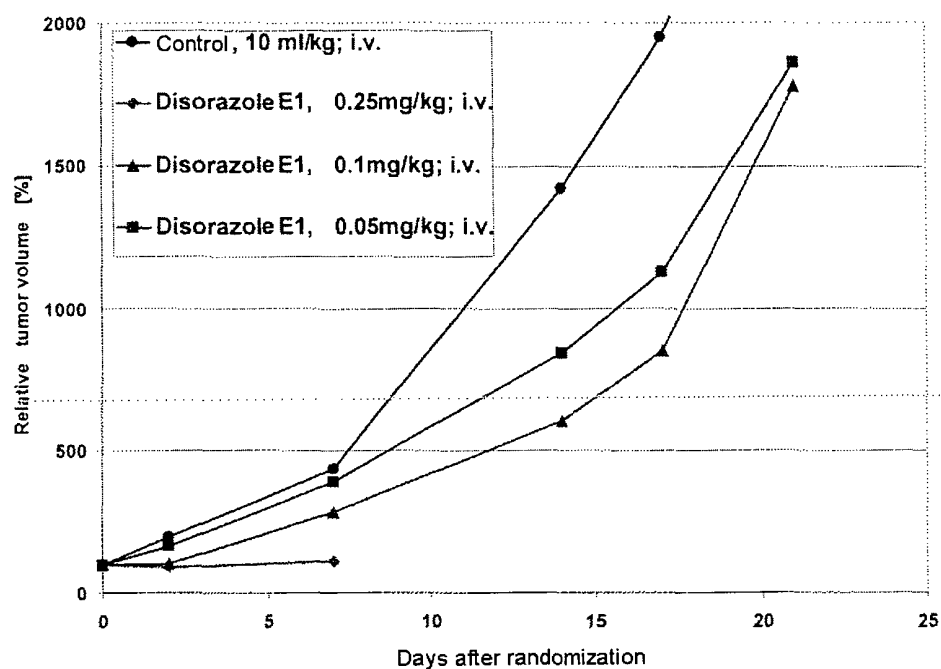
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14 The *in-vivo* activity of the compounds according to the invention was tested on  
15 human and murine xenograft models. In the therapy experiment, with NCI-  
16 H460 tumor xenograft-bearing nude mice, it was possible for disorazole E1  
17 administered i.v. to produce a significant reduction of the tumor growth even at

1 doses which produced no significant weight decrease or perhaps even  
2 mortality.

3  
4 Figure 1: *In-vivo* treatment experiment with disorazole E1 in the NCI-H460  
5 tumor xenograft

6 Model on nude mice



7

8

9 Disorazole E1 (D-42805): 0.25mg/kg; i.v.: day 0, 7; 8 dead (day  
10 11,12,13)

11 Disorazole E1 (D-42805): 0.1mg/kg; i.v.: day 0,7,14; no cases of  
12 death

1 Disorazole E1 (D-42805): 0.05mg/kg; i.v.: day 0,7,14; no cases of  
2 death  
3 Control: 0.9% strength saline solution containing 3.3% DMSO, 10 ml/kg;  
4 n = 8 animals/group  
5

## 6 **Example 12 AMES test**

7

8 For the estimation of possible side effects, disorazole E1 was investigated for  
9 mutagenicity in a fluctuation assay against the mutant strains TA98 and TA100  
10 of the bacterium *Salmonella typhimurium* at three concentrations (2.5; 5 and  
11 10µM). The mutagenicity investigations were further carried out in the presence  
12 of the rat liver enzyme S9.  
13

14 The results are compiled in table 5 below:

Compound	Conc. [µM]	AMES TA98 without S9	AMES TA98 with S9	AMES TA100 without S9	AMES TA100 with S9
Disorazole E1	10	inactive	inactive	inactive	inactive
Disorazole E1	5	inactive	inactive	inactive	inactive
Disorazole E1	2.5	inactive	inactive	inactive	inactive

15

16 Table 5: Investigation of **disorazole E1** for mutagenicity

17

1 Disorazole E1 shows no effects under the assay conditions described in the  
2 abovementioned concentrations, it is thus AMES test-inactive.

3

4 **Example 13 Influence on protein biosynthesis and nonproliferating cells**

5

6 For the estimation of the possible side-effect potential, the influence of  
7 disorazole E1 on nonproliferating cells and on the protein biosynthesis was  
8 investigated (table 6).

Substance	Conc. [µM]	Surviving cells, primary human hepatocytes <sup>1</sup>	Protein synthesis <sup>2</sup>
		Average, % of control	Average, % of control
Disorazole E1	1	119.6	95.9
<sup>1)</sup> Tested with alamarBlue, human primary hepatocytes, n = 3;			
<sup>2)</sup> Tested via the incorporation of <sup>14</sup> C-methionine, human hepatocellular carcinoma cells (HepG2), n= 2;			

9

10

11 Table 6: Influence of disorazole E1 on nonproliferating cells and on the  
12 protein biosynthesis

13

14 The results of table 6 show that disorazole E1 neither acts negatively on the  
15 protein biosynthesis nor on the survival of nonproliferating cells.

16